JOURNAL OF VIROLOGY, Nov. 1982, p. 752–754
0022-538X/82/110752-03$02.00/0
Copyright © 1982, American Society for Microbiology
Vol. 44, No. 2

Chromosomal Localization of the Moloney Sarcoma Virus Mouse Cellular (c-*mos*) Sequence

DAVID SWAN,[1]* MARIANNE OSKARSSON,[2] DEBRA KEITHLEY,[1] FRANK H. RUDDLE,[3] PETER D'EUSTACHIO,[3] AND GEORGE F. VANDE WOUDE[2]

Laboratory of Cellular and Molecular Biology[1] and Laboratory of Molecular Oncology,[2] National Cancer Institute, Bethesda, Maryland 20205, and Department of Biology, Yale University, New Haven, Connecticut 06511[3]

Received 1 June 1982/Accepted 6 July 1982

The Moloney sarcoma virus-specific *onc* gene, referred to as v-*mos*, was used as probe to hybridize to restricted DNAs from various mouse-Chinese hamster hybrid cell lines. These hybrid cells contain, in addition to all of the Chinese hamster chromosomes, various numbers (less than a full complement) of mouse chromosomes. Comparison of the presence or absence of the mouse cellular *mos* gene with the known karyotype in each of the hybrid cell lines allows us to conclude that the *mos* gene is on mouse chromosome 4.

The acute transforming retroviruses have acquired cellular sequences, called *onc* genes, that are responsible for their ability to cause rapid neoplastic transformation. *onc* genes are usually found in few or single copies in the host genome and are highly conserved among divergent animal species (3). The role of c-*onc* genes in normal cells is unknown, but it is presumed that they serve essential purposes since they have been conserved over long periods of evolutionary time. It is of interest to know the chromosomal location of the c-*onc* genes to determine (i) whether all of the known c-*onc* genes are linked on one chromosome or scattered throughout the genome; (ii) whether neighboring cellular genes are coordinately expressed during transformation; and (iii) the connection, if any, between known chromosomal aberrations in naturally occurring malignancies (12) and the location of c-*onc* genes. In this paper, we describe a series of experiments designed to identify the chromosomal location of the cellular homolog of the Moloney murine sarcoma virus (M-MSV) transforming gene (v-*mos*).

We used a nick-translated *mos*-specific probe isolated from a cellular DNA homolog (4, 17) to detect the corresponding c-*mos* sequence in DNA extracted from various mouse-Chinese hamster hybrid cell lines. The c-*mos* sequence is highly conserved among animal species (9), and we show that both Chinese hamster and mouse genomic DNA possess sequences homologous to the v-*mos* probe (Fig. 1, lanes 4 and 5, respectively). In *Sst*I-digested genomic DNA, the Chinese hamster c-*mos* fragment is 4.3 kilobase pairs (kb) in size, and the mouse fragment is 6.0 kb (14). This enzyme was used to digest genomic DNA from Chinese hamster-mouse hybrid cell lines (Fig. 1). The hybrid cell lines tested (Table 1) contained a full complement of Chinese hamster chromosomes, but they segregated various numbers of mouse chromosomes. Thus, v-*mos* hybridization to the hybrid cell DNA showed the presence of the hamster 4.3-kb fragment in every case. However, the 6.0-kb mouse fragment was absent in 4A6-4, EE1-1, ECm4e, and 132Az2 (Fig. 1, lanes 3, 8, 9, and 10) but was present in 2A1, 2A2, BEM1-6, and 6D3Az (Fig. 1, lanes 1, 2, 6, and 7). Comparison of the presence of the 6.0-kb fragment with the chromosome content of the hybrid cells shows complete concordance with the presence of mouse chromosome 4. Hybrid cell line 6D3Az (Fig. 1, lane 7) has only mouse chromosome 4 and still contains the mouse c-*mos* structural gene (Table 1). These results clearly demonstrate that the c-*mos* gene is present on mouse chromosome 4.

There are now more than 15 known viral *onc* genes (6). It will be of interest to see whether others are also genetically linked to the mouse chromosome 4. Other markers which map on chromosome 4 are the Friend virus 1 locus (*Fv-1*). This locus possesses a determinant for susceptibility to infection by murine retroviruses. The *Fv-1* locus has been shown to map within 0.6 centimorgans of hexose-6-phosphate dehydrogenase (*Gpd-1*) on mouse chromosome 4 (15, 16). It is not known, however, whether there is any connection between *Fv-1* and any of the known c-*onc* genes.

Tumor induction by retroviruses, in some cases at least, has been shown to result from retroviral insertion adjacent to a cellular *onc*

TABLE 1. Mouse chromosome content in hybrid cell lines tested for the presence of c-*mos*[a]

Lane[b]	DNA	Mouse chromosome no.[c]																			Hybridization to *mos*
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	X	
1	2A1	62	85		38		50	69	27	58	35		81	4	62	77	73	69	19	69	46	+
2	2A2	53	90	22	43	2	69	69	33	65	51		73	22	82	90	80	82	55	82	41	+
3	4A6-4	48	79				79	79								154	86		1	107	107	−
4	Chinese hamster	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
5	Mouse	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	BEM1-6	61	103	94	97		197[d]	13	87	23	27		74	77	103	174	87	87	55	126	185[d]	+
7	6D3Az				14																	+
8	EE1-1		89						85				85					10	18			−
9	ECm4e														100[d]	100[d]						−
10	132Az2		18					63								63		1	75[d]	75[d]		−

[a] Mouse chromosomes were identified by trypsin-Giemsa-Hoechst staining of metaphase spreads from hybrid cells.
[b] Lane number refers to lanes in Fig. 1.
[c] Numbers indicate the mean number of copies of the chromosome per 100 cells control DNAs were run in lanes 4 and 5. The presence or absence of mouse chromosomes is indicated by + and −, respectively.
[d] Includes copies of chromosomes present as translocations.

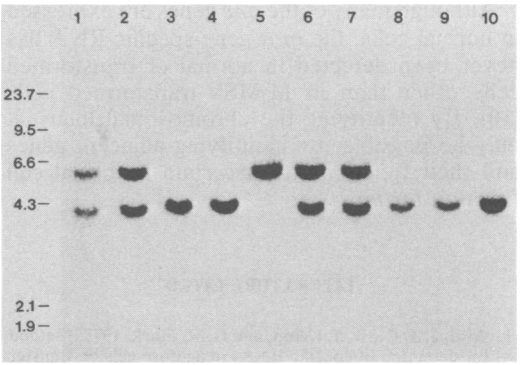

FIG. 1. Hybridization of a cloned c-*mos* probe to DNA from mouse-hamster hybrid cell lines. DNA was extracted from the cell lines identified in Table 1 and from mouse and hamster spleens. Molecular weights indicated on the left were obtained by running a *Hin*dIII digest of phage λ DNA. Hybrid cell DNA and mouse and hamster control DNAs were digested with *Sst*I restriction endonuclease and electrophoresed on 1% agarose gels in 40 mM Tris-hydrochloride (pH 7.5)–20 mM sodium acetate–1 mM EDTA–0.5 μg of ethidium bromide per ml. DNA was transferred to diazobenzyloxymethyl paper (1) and hybridized to a nick-translated *Ava*I-*Hin*dIII *mos*-specific fragment derived from pMS1 (4). Hybridization was performed at 42°C for 15 h in 50% formamide—5× SSC (1× SSC: 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% Ficoll–0.1% bovine serum albumin–0.1% polyvinyl pyrrolidone–20 mM sodium phosphate (pH 6.5)–100 μg of sheared, denatured salmon sperm DNA per ml–10% sodium dextran sulfate–10^7 cpm of ^{32}P-labeled *mos* probe per ml (labeled to ~700 cpm/pg). Filters were washed three times at room temperature in 0.1× SSC–0.1% SSC, then twice at 45°C in the same buffer. Bands indicate Chinese hamster DNA (lane 4); mouse DNA (lane 5); 2A1 (lane 1); 2A2 (lane 2); 4A6-4 (lane 3); BEM1-6 (lane 6); 6D3Az (lane 7); EE1-1 (lane 8); ECm4e (lane 9), and 132Az2 (lane 10).

gene (11, 13). Transcriptional control sequences supplied by the long terminal repeat sequences of the retrovirus effect higher levels of *onc* gene expression. It has also been demonstrated that cellular *onc* genes can be activated to cause cellular transformation by generating hybrids in vitro between a viral long terminal repeat sequence and the *onc* gene (4, 7).

It is not known how nonviral oncogenesis is initiated, but there is evidence that it is elicited by (i) mutations (2, 8); (ii) chromosomal rearrangements (12); or even (iii) reversible epigenetic events (5). T cell leukemias are induced by various agents, including Moloney leukemia virus, X rays, and chemical carcinogens (18), and all exhibit chromosomal abnormalities usually involving chromosomes 15, 12, and 6. Chromosome 4 has never been implicated in such aberrations.

Although many of the *onc* genes are expressed in normal cells, the *mos* gene-specific RNA has never been detected in normal or transformed cells other than in M-MSV-transformed cells (10). By identifying the chromosomal locus, it may be possible, by identifying adjacent genes and their functions, to ascertain a normal cell function for *mos*.

LITERATURE CITED

1. **Alwine, J. C., D. J. Kemp, and G. R. Stark.** 1977. Method for detection of specific RNAs in agarose gels by transfer to diazobenzyloxymethyl-paper and hybridization with DNA probes. Proc. Natl. Acad. Sci. U.S.A. **74**:5350–5354.
2. **Ames, B. N.** 1979. Identifying environmental chemicals causing mutations and cancer. Science **204**:587–593.
3. **Bishop, J. M.** 1981. Enemies within: the genesis of retrovirus oncogenes. Cell **23**:5–6.
4. **Blair, D. G., M. Oskarsson, T. G. Wood, W. L. McClements, P. J. Fishinger, and G. F. Vande Woude.** 1981. Activation of the transforming potential of a normal cell sequence: a molecular model for oncogenesis. Science **212**:941–943.
5. **Cairns, J.** 1981. The origin of human cancers. Nature (London) **289**:353–357.
6. **Coffin, J. M., H. E. Varmus, J. M. Bishop, M. Essex, W. D. Hardy, Jr., G. S. Martin, N. E. Rosenberg, E. M. Scolnick, R. A. Weinberg, and P. K. Vogt.** 1981. Proposal for naming host cell-derived inserts in retrovirus genomes. J. Virol. **40**:953–957.
7. **DeFeo, D., M. A. Gonda, H. A. Young, E. H. Chang, D. R. Lowy, E. M. Scolnick, and R. W. Ellis.** 1981. Analysis of two divergent rat clones homologous to the transforming gene of Harvey murine sarcoma virus. Proc. Natl. Acad. Sci. U.S.A. **78**:3328–3332.
8. **Epstein, S. S., and J. B. Swartz.** 1981. Fallacies of lifestyle cancer theories. Nature (London) **289**:127–130.
9. **Frankel, A. E., and P. J. Fischinger.** 1977. Rate of divergence of cellular sequences homologous to segments of Moloney sarcoma virus. J. Virol. **21**:153–160.
10. **Gattoni, S., P. Kirschmeier, I. B. Weinstein, J. Escobedo, and D. Dina.** 1982. Cellular Moloney murine sarcoma (c-mos) sequences are hypermethylated and transcriptionally silent in normal and transformed rodent cells. Mol. Cell. Biol. **2**:42–51.
11. **Hayward, W. S., B. G. Neel, and S. M. Astrin.** 1981. Activation of a cellular *onc* gene by promoter insertion in ALV-induced lymphoid leukosis. Nature (London) **290**:475–480.
12. **Klein, G.** 1981. The role of gene dosage and genetic transposition in carcinogenesis. Nature (London) **294**:313–318.
13. **Neel, B. G., W. S. Hayward, H. L. Robinson, J. Fang, and S. M. Astrin.** 1981. Avian leukosis virus-induced tumors have common proviral integration sites and synthesize discrete new RNAs: oncogenesis by promoter insertion. Cell **23**:323–334.
14. **Oskarsson, M., W. L. McClements, D. G. Blair, J. V. Maizel, and G. F. Vande Woude.** 1980. Properties of a normal mouse cell DNA sequence (*sarc*) homologous to the *src* sequence of Moloney sarcoma virus. Science **207**:1222–1224.
15. **Rowe, W. P., and H. Sato.** 1973. Genetic mapping of the Fv-1 locus of the mouse. Science **180**:640–641.
16. **Taylor, B. A., H. G. Bedigian, and H. Meier.** 1977. Genetic studies of the *Fv-1* locus of mice: linkage with *Gpd-1* in recombinant inbred lines. J. Virol. **23**:106–109.
17. **Vande Woude, G. F., M. Oskarsson, L. W. Enquist, S. Nomura, M. Sullivan, and P. J. Fischinger.** 1979. Cloning of integrated Moloney sarcoma proviral DNA sequences in bacteriophage lambda. Proc. Natl. Acad. Sci. U.S.A. **76**:4464–4468.
18. **Wiener, F., S. Ohno, J. Spira, N. Haran-Ghera, and G. Klein.** 1978. Cytogenetic mapping of the trisomic segment of chromosome 15 in murine T-cell leukemia. Nature (London) **275**:658–660.